Leptospirosis in cattle in South Africa

Dr. J.H. Vorster, BVSc, MMedVet (Path)
Vetdiagnostix Veterinary Pathology Services,
Pobox 13624, Cascades, PMB, 3202
E-mail: vorsterj@ampath.co.za
Cell no: 082 820 5030

Dr. P.H. Mapham, BVSc (Hon)
Veterinary House Hospital
339 Prince Alfred Road, PMB, 3201
E-mail: rickm@iafrica.com
Cell no: 082 771 3227

Introduction

Leptospirosis, an infectious disease of both animals and humans, is caused by infection with bacteria of the genus *Leptospira*. Leptospirosis in cattle may be manifested by different clinical syndromes such as a transient febrile response, a life threatening haemolytic crisis, chronic interstitial nephritis, mastitis, abortions, stillbirths and reproductive failure in cattle. Leptospirosis is an important zoonotic disease.

The genus *Leptospira* consists of many species that can only be distinguished by genotyping into antigenically related serogroups, which are further grouped into different serovars. This is accomplished on the basis of the antigenic composition of the bacteria, determined by agglutination and cross-agglutinin absorption tests. This article deals primarily with leptospirosis in cattle.

Epidemiology

Animal hosts are categorized into maintenance and incidental hosts. Maintenance hosts are usually infected easily and develop mild or no clinical signs. Organism may then localize in their kidneys and these animals may then serve as source of infection for incidental hosts. Maintenance hosts may include several species of wildlife but also domestic animals and livestock. Maintenance hosts identified worldwide include rats, bats, jackals, foxes, oppussums, skunks, wild boar, deer and raccoons. Domestic livestock identified as maintenance hosts are for example pigs (*L. pomona*), horses and pigs (*L. bratislava*) and cattle (*L. hardjo*). In South Africa serological investigation and attempts at culture in 19 different species of rodents failed to reveal any significant numbers of maintenance hosts. A few reedbuck, bushpig, black wildbeest, nyala and rhinoceroses had positive antibody titres against a few serovars. The incidence in maintenance hosts may be high as transmission between these hosts may be very efficient via direct contact with infected urine, placental fluids or milk.
Transmission may also take place transplancently, venereally, during coitus, artificial insemination and organisms may be preserved in semen.

In contrast incidental hosts are not important reservoirs of infection and the incidence of infection is low. They may exhibit severe clinical signs once they are infected. Transmission between incidental hosts is far less common. Infection of incidental hosts is more commonly indirect through contact with areas contaminated with urine of maintenance hosts.

Different serovars may be prevalent in different geographical areas and may be associated with different maintenance hosts greatly influencing the epidemiology in many different parts of the world. The number of carrier animals in a population, the level of shedding of organisms via urine into water-born wastes such as slurry and run-off water may all influence the rate of spread of the infection. The survival of leptospires in the environment is highly dependent on temperature and humidity and dry conditions and pH values below six or exceeding eight are very detrimental to their survival outside hosts. These bacteria have been reported to survive for at least 183 days in wet soil. In contrast they may survive for less than 30 minutes in air-dried soil. Stagnant water may remain contaminated by live leptospires for prolonged periods. Survival time for organisms in acid soils with a low moisture content may be as long as 24 days.

Leptospirosis in cattle in South Africa has therefore mostly been reported from areas with relatively high rainfall such as Mpumalanga, KwaZulu Natal and the coastal area of the Eastern Cape provinces. However, it has also been diagnosed in the drier Limpopo and North West provinces. As high temperatures and dry conditions reduce the environmental survival of the organisms spring, autumn and early winter are associated with highest prevalence in temperate climates.

Reliable estimates of the prevalence of leptospirosis are not freely available due to the difficulty in diagnosing the condition. A few serological surveys have been carried out in South Africa. One such recent serological survey was conducted in cattle originating from rural communities of the province of KwaZulu-Natal (KZN) in South Africa and revealed a prevalence of 19.4% with a 95% confidence interval of 14.8-24.1%. The southeastern regions of the province showed a higher prevalence than other areas of the province. *Leptospira interrogans* serovar *pomona* occurred most frequently but serovars *tarrasovi, bratislava, hardjo, canicola* and *icterohaemorrhagica* were also frequently identified. Serovar *hardjo* has been reported as the most important serovar worldwide in cattle. This seems to be true in Southern Africa as well, whilst *L. pomona* has been reported as a cause of sporadic infections. Others serovars identified in South Africa in cattle include *L. pomona, L. mini, L. canicola* and *L. hardjo*. Serovars *L. tarassovi, L. mini* and *L. hardjo* were identified in semen. In South Africa the occurrence of disease does not seem to be much different between communal and commercial farms.
Clinical signs and post mortem findings

Most serovars of *Leptospira* sp which infect cattle cause acute, subacute or inapparent disease. The severity of clinical signs is associated with the serovar and species of animal affected. In cattle *L. hardjo*, for example, is reported to show subtle clinical signs with a renal carrier state being a common sequel. Reduced reproductive efficiency and milk production have been reported together with abortions, stillbirths and weak calves.

Following on indirect transmission, usually by water or mud contaminated with infected urine, particularly on abraded skin, or skin softened by prolonged immersion in water, leptospires enter the blood stream. Organisms localize in the liver where primary replication takes place, followed by release of organisms into the blood, resulting in a leptospiraemia. This phase coincides with a febrile reaction and malaise. Thereafter organisms again localize in organs such as the lung, brain, kidneys, eyes or the pregnant uterus. Clinical signs are seen after an incubation period varying from 2 to 16 days, and expression of signs are largely dependent on the site of localization.

Animals with acute severe leptospirosis may show clinical signs of fever, anorexia, malaise, leptospiruria and haemoglobinuria, icterus and anaemia and is commonly associated with *L. pomona*. After infection the organism may multiply in the liver leading to fever lasting for 1 to 5 days. Relapses may occur recurrently for 2 to 3 months. Infected calves may show signs of diarrhoea and may die within 1 to 7 days. Infected cows may show reduced milk yields and give birth to week or immature calves. Cows which abort may show mild clinical signs, develop a non inflammatory mastitis and short term agalactia.

Subacute infections may present with fever, mild haemoglobinuria and mastitis. Subclinical or inapparent infections are usually only detected when it results in abortions (eg *L. pomona* in South Africa) or when diagnosed through routine serological screening.

**Macroscopic post mortem** lesions which may be highly suggestive, but not diagnostic, may include icterus, anaemia, splenomegaly, widespread haemorrhages (mucosal and subcutaneous), hepatomegaly, pulmonary oedema and acute or chronic interstitial nephritis. Foetal pathology is non-specific and foetuses may be fresh or autolysed. No significant lesions are seen in the placenta.

During 2008 a Friesland cow was presented for post mortem examination showing signs of severe icterus and haemoglobinuria as the most striking macroscopic lesions (see figure 1 below). Subsequently *Leptospira* sp organisms were identified in the kidneys by immunohistochemistry (see below).
Diagnosis

In most cases the diagnosis of leptospirosis may be difficult and is usually the greatest challenge facing a veterinarian in the face of a suspected outbreak.

Isolation of these bacteria is demanding, time-consuming (may be 10 days to 26 weeks depending on serovar). A short transit time between sampling and culture is crucial. Samples should be stored at 4°C and should reach the laboratory within three hours. The use of specific transport media, liquid culture media or direct inoculation of samples on to culture media may increase the changes of success. The use of diuretics (furesomide) may also aid in a positive urine culture in live animals.

Isolation of the organisms from organ samples such as the liver, lung, brain, adrenal gland and kidney, collected from dead animals and aborted foetuses; and body fluids such as blood, milk, cerebrospinal, thoracic and peritoneal fluids, collected from clinically infected animals, and foetuses, may confirm a diagnosis of acute clinical disease. In the case of a foetus it would confirm chronic infection of the dam. Isolation of organisms from the kidney, urine or genital tract of animals not showing clinical signs may be diagnostic of a chronic carrier state. The kidney is the organ of choice for the diagnosis of chronic carriers. A single negative culture does not guarantee a true negative result and three negative cultures over a period of three weeks is advised before considering an animal as negative. It may be useful to identify which serovar/s are present within a particular group of animals, an animal species, or a geographical region. Due to all these pitfalls culture it is not always attempted or not always a viable option.
**Dark field microscopy** of urine and blood samples may be attempted but is generally of little practical use.

**Polymerase chain reaction (PCR)** technology may provide a diagnosis even if the tissues contain very few bacteria. These techniques may be serovar specific but may also be prone to a low specificity (false positive results). Samples similar to that described for virus isolation should be collected. Samples should also be stored at 4°C and should reach the laboratory as soon as possible.

**Serological investigations** may be carried out on paired acute and convalescent serum samples. To a large extent this may currently form the basis of diagnostic investigation, or laboratory confirmation, in most suspected outbreaks in South Africa. However, it has some drawbacks. Cross reactions between serovars, vaccination against certain serovars and prevalent titres in endemic areas may complicate the interpretation of results. Antibodies may appear very shortly after infection and may persist for weeks to years, but may also drop to undetectable levels in chronic carrier animals. Anamnestic responses in mature animals have been described.

The microscopic agglutination test (MAT) is used primarily as a herd test and at least ten animals, or 10% of the herd, whichever is greater, should be tested to be of benefit.

A four-fold rise in antibody titres in paired acute and convalescent serum samples is diagnostic of acute infection in an individual animal. MAT is limited to diagnose chronic infection in individual animals, and to diagnose endemic infections in herds. Several enzyme-linked immunosorbent assays (ELISAs) assays have been developed to replace the MAT and is very useful to test large numbers of samples. These are usually very sensitive but lack the serovar specificity of the MAT.

During necropsy a full set of organ samples including brain, lung, heart, liver, spleen, kidneys, lymph nodes, and gastrointestinal samples should be collected in 10% formalin to confirm the presence of lesions consistent with leptospirosis and to exclude any other disease condition. The kidney should be an organ of choice in which interstitial nephritis may be seen. Aborted foetuses/stillbirths should be subjected to a full post mortem examination. **Warthin Starry staining** of tissues can also be attempted but these tests lack both sensitivity and specificity, and needs to be used in conjunction with PCR or bacterial isolation.

Organisms may be demonstrated by **immunohistochemistry** but the efficacy of these tests is dependent on the number of organisms present within the tissue, and possibly prior treatment. Another drawback is the failure of this technique to identify the organism down to serovar level. The authors recently demonstrated the presence of *Leptospira sp* organisms in the kidneys of animals showing macroscopic lesions consistent with those described above by means of IMP staining (figure 2).
In these animals the histological lesions were mainly characterized by very mild multifocal lesions of interstitial nephritis with a lymphoplasmacytic to mild purulent cellular infiltrate. Hepatic lesions were characterized by mild variable periacinar degeneration and necrosis, cholestasis and hydropic degeneration.

Leptospirosis in South Africa

Subsequent to the diagnosis made in the above case report, owners were requested to submit blood smears from dead cattle that showed icterus AND/OR haemoglobinuria. Where Babesia sp or Anaplasma sp parasites were not readily identified the cases were provisionally categorised as leptospirosis. Deaths showing icterus only were categorised as gallsickness, while those with haemoglobinuria only were categorised as redwater - and where possible the diagnosis was supported by the blood smear. Blood smears were not always conclusive either way, for various reasons, so the macroscopic classification was taken as the presumptive diagnosis unless the blood smear contradicted this. In this way 28 gallsickness cases, 57 redwater and 102 leptospirosis cases were identified. This categorisation is obviously crude and inaccurate, however, the seasonal variation of all three conditions showed the highest prevalence in the autumn months which may be expected again from all three diseases.
Categorising the diagnosis by breed is shown in the table below:

<table>
<thead>
<tr>
<th>BREED</th>
<th>GALLSICKNESS</th>
<th>LEPTOSPIROSIS</th>
<th>REDWATER</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRIESLAND</td>
<td>13</td>
<td>92</td>
<td>25</td>
</tr>
<tr>
<td>OTHER</td>
<td>15</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>TOTAL</td>
<td>28</td>
<td>102</td>
<td>57</td>
</tr>
</tbody>
</table>

Significantly more Friesland animals were categorised as leptospirosis (92 vs 10) than in other breeds. Cases categorised as leptospirosis were not necessarily younger calves as reported in an Israeli feedlot by Yeruham et al.

**Conclusion**

These findings raise questions on the significance of *Leptospira* sp infection in livestock in South Africa, possible serovars involved, possible maintenance hosts involved, and also many different questions on various epidemiological aspects. Unfortunately the difficulty and cost incurred in making an accurate diagnosis was prohibitive and further research in this diagnosis will be necessary in order to fully evaluate the real prevalence of Leptospirosis in South African dairies, the impact in dairy and feedlot health and the possible implications for human health.
Further reading: